

Association of the Leptin Receptor Q223 (rs1137101) Polymorphism with Infertility in Iraqi Patient Women

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Abstract

Obesity and Type2 diabetes mellitus regarded chronic metabolic disease and main causes of infertility in women. Obesity lead to increase of Leptin hormone level, A type I cytokine receptor called LEP-R serves as a receptor for the hormone leptin, which is only found in fat cells. The importance of this function is made clear by the leptin receptor deficiency condition, which is characterized by elevated leptin levels in the blood plasma and, as a result, by constant hunger and overeating that result in obesity and infertility. The LEPR gene, which is found in humans, encodes a protein that on 1p3.3. Ninety Iraqi women were enrolled as a samples in this study collected from women's hospital in Karbala city and teaching hospital and the women and children hospital of Al-Diwaniyah province from October 2020 to January 2021. Sixty infertility women were suffered from obesity and T2DM and 30 were apparently healthy considered as a control. Study data to analyze by chi square and odds ratio and p-value test used to detect significance of the means. Genotype result rs1137101 related with GG homozygous not associated with obesity compared with diabetes (16.66 versus 13.33%; $X^2 = 0.131$) and obesity compare with control (16.66 versus 30%; $X^2 = 1.49$). Result of rs1137101 related with AG heterozygous not associated with obesity compared with diabetes (73.33 versus 80%; $X^2 = 0.373$), and obesity compare with control (73.33 versus 60%; $X^2 = 1.2$). Result of rs1137101 related with AA homozygous not associated with obesity compared with control (10 versus 10 %; $X^2 = 0$), and diabetes compare with control (6.66 versus 10%; $X^2 = 0.218$). The result of this study concluded that rs1137101 revealed that GA heterozygous, GG and AA homozygous are not associated of incidence infertility that suffered from diabetes and obesity in Iraqi patient women.

Keywords: LEPR, Infertility, Obesity, Diabetes.

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INTRODUCTION

The failure to conceive spontaneously after a year of routine, unprotected sexual activity is referred to as infertility (Chowdhury, Cozma and Chowdhury, 2017). The majority of the time, infertility is a form of subfertility in which one in seven couples requires medical assistance to become pregnant (Taylor, 2003). Anovulation, clogged fallopian tubes, endometriosis, or uterine abnormalities can all be symptoms of female infertility (Adamson and Baker, 2004; Foster et al, 2008). A woman's pregnancy, often referred to as gestation, is the time during which one or more children develop inside of her (Fok, Chan and Yuen, 2005), Multiple pregnancies result in multiple offspring, such as twins (Wylie, 2005). Pregnancy often results via sexual coitus, however it can also be brought on by using assisted reproductive technology (Shehan 2016).

Obesity is a medical condition when there is too much body fat, which could be harmful to one's health. (Luppino et al, 2010).

Obesity is linked to a number of illnesses and ailments,

especially heart problems, type 2 diabetes, infertility, and some types of cancer. (Yazdi, Clee and Meyre, 2015). Obese women, especially those with abdominal fat, have a difficult time getting pregnant and have a low likelihood of receiving infertility therapy. The association between obesity and PCOS also makes obese women more likely to have infertility. (Priscilla et al, 2012). Obese infertile women have been shown in studies to have systemic hyperestrogenemia, hyperinsulinemia, and ovarian dysfunction due to early follicular atresia and anovulation. (Gambeniri et al, 2019). Obesity has been shown to inhibit oocyte quality and maturation as well. (Yang et al, 2012), but also embryo development. It's one of causes of female infertility because the obesity lead to increase of Leptin hormone level (Pratibha et al, 2107). The hormone leptin (LEP), which is largely produced by adipocytes and enterocytes in the small intestine and is located on chromosome 7q32.1, helps to manage energy balance by reducing appetite. The protein leptin has 167 amino acids and a molecular weight of around 16 kD. Friedman identified the protein's DNA sequence in 1994. (Flier, 2018). In addition to other regions of the hypothalamus and dopaminergic neurons in the ventral tegmental area,

leptin acts on cell receptors in the bent and ventromedial nuclei to mediate feeding. (Brennan, 2006; Bouret, Levin and Ozanne, 2015). Leptin receptor is a type I cytokine receptor also known as LEP-R or OB-R. (Cirillo et al, 2008), a protein that is produced in humans by the 1p31.3-located LEPR gene. (Tartaglia, 1995), LEP-R serves as a receptor for the hormone leptin, which is only found in fat cells. Additionally, CD295 has been assigned to LEP-R. (cluster of differentiation 295). Its intracellular, trans-membrane, and extracellular parts are all located within the cell membrane (protein regions). Mutations in this gene have been correlating with obesity and pituitary dysfunction (Illangasekera et al, 2020). When leptin interacts to leptin receptors, particularly those in the hypothalamus, chemical signals are created that induce a feeling of satiety and reduce hunger. Increasing the serum level of leptin results in a decrease in leptin receptor. The importance of this function is demonstrated by the leptin receptor deficiency condition, which is characterized by elevated leptin levels in blood plasma and, as a result, by constant hunger and overeating that result in obesity. (Olga et al, 2019). Hypogonadism, delayed or missing puberty, and infertility are all associated with a lack of leptin receptors. (Carol and Darshana, 2013). Increase level of leptin hormone lead to obesity and obesity cause increase of insulin level in blood and insulin resistance also a known diabetes type two (Winick, Stoffel and Friedman, 1996). Influence of the rs1137101 leptin receptor gene polymorphism, which results in a non-conservative A to G substitution at codon 223 and a glutamine to arginine amino acid shift, on the development of the two kinds of diabetes mellitus. (Illangasekera et al, 2020). This functional variation weakens leptin signaling by reducing leptin binding. (Quinton et al, 2001).

This study was aimed to determineThe genotypes that are related to the occurrence of infertility in the LEPR genes under study.

MATERIAL AND METHODS

Sample Collection

Ninety samples from women were used in this study infected with Infertility that suffered from diabetic and obesity, and without Infertility, Ninety Iraqi women were enrolled as a samples in this study collected from women's hospital in Karbala city and teaching hospital and the women and children hospital of Al-Diwaniyah province, Iraq. Total of 90 samples were divided into three groups. The first group included 30 samples from infertility that suffered from obesity, second group includes 30 samples from infertility that suffered from T2DM, the third group includes 30 samples from apparently healthy women considered as a control.

Genotyping and Genomic DNA Isolation

DNA extraction

The blood samples were collected for DNA extraction (using a kit from Intron Biotechnology in Korea), according to the

manufacturer's instructions, and were then stored at -20C. DNA purity and concentration were assessed using a UV spectrophotometer.

Primers design

Table (1): The Tetra ARMS-PCR primers with their sequence and PCR size product for rs1137101 A/G of LEPR polymorphism.

Primer	Sequence (5'-3')	Tm (C°)	PCR bandSize (Pb)
FI Allele- G	GAAAATCACATCTG G- TGGAGTAATTTTACG	65	226
RI Allele- A	GGGCTGAACTGACA TT-AGAGGTGCCT	68	201
FO	AGGCCTGAAGTGTT A-GAAGATTCACCTC	66	
RO	CATTCTAGAAGCCAC -TCTTAATACCCCA	66	

ARMS-PCR master mix preparation

Polymorphism analysis was performed in duplicate according to protocols based on the tetra-primer amplification refractory mutation system (ARMS)-polymerase chain reaction (PCR) using 2 primer pairs to amplify the 2 alleles of SNP, respectively, in a single PCR reaction. The PCR primers used were forward inner primer (G allele) 5 - GAAAATCACATCTGGTGGAGTAATTTTACG, reverse inner primer (A allele) 5- GGGCTGAACTGACATTAGAGGTGCCT, forward outer primer 5 -AGGCCTGAAGTGTTAGAAGATTCACCTC and reverse outer primer 5 - CATTCTAGAAGCCACTCTTAATACCCCA. Each PCR reaction was performed in a total volume of 25µl, adding 12.5 µl of master mix, 0.5 µl of an isolated DNA solution, and nuclease-free water to the tubes 4 µl, 1 µl MgCl₂, along with 5 µl of outer primer, and adding 2 µl of inner primer. PCR cycling conditions for the assay were 95C° for 5 minutes, followed by 35 cycles of touchdown reactions at 95C° for 30 second for the first cycle, and then continuing at 61C° for 30 second in the annealing step of the remaining cycles with extension at 72C° for 30 second and a final extension step at 72C° for 10 minute.

STATISTICAL ANALYSIS

The Statistical Package for Social Sciences (SPSS version 26, Inc., Chicago, IL, USA) and the Microsoft Excel Worksheet were used to conduct the statistical analysis. Analysis was done on the current study's findings and case studies. Odd ratio, 95% confidence interval, and values less than 0.05 (P0.05) were used to assess the significance of the P-value. (Sorlie, 1995).

RESULTS

The results of this study show distribution of Leptin receptor (rs1137101) Polymorphism was diagnosed by Tetra ARMS-PCR. There are many genotypes; AG, AA and GG. The wild type homozygote genotype was showed only A allele amplification at 201 bp product size. The mutant type homozygote genotype was showed only G allele amplification at 226 bp product size. Whereas, the heterozygote genotype was showed A and G allele's amplification between 201,226 bp product size respectively.

Genotypic and Alleles Analysis for studied genes in Patient groups and control groups, The distribution of infertility with groups was shown in Figure (1), according to Leptin receptor (rs1137101) genotypes and alleles was showed in table (2).

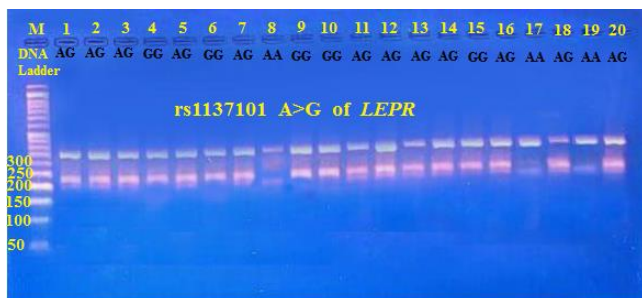


Figure 1. Electrophoresis for PCR product of LEPR gene (rs1137101). Lanes 4,6,9,10,15 (GG) 226 bp mutant homozygous; Lanes 1,2,3,5,7,11,12,13,14,16,18,20 (AG) between (201-226bp) heterozygous ; Lanes 8,17,19 (AA) 201 bp wild type homozygous. Agarose 1.5 % concentration, using a 100 bp ladder as molecular weight marker at 75 voltages for 45 min. stained with ethidium bromide. (Abbreviation: L, DNA Ladder).

DISCUSSION

The most prevalent endocrinopathy in women of childbearing age is obesity, which affects both the reproductive and metabolic systems. Ovulatory dysfunction, aberrant hormone levels, hyperandrogenemia, and hyperinsulinemia are all present in obese women. (Franks, McCarthy and Hardy, 2006). Obesity lead to increase of leptin hormone level which synthesis by adipose cells and enterocytes and cause systemic hormonal imbalance whereby obesity one of main causes of infertility and type-2 diabetic. A type I cytokine receptor called LEP-R serves as a receptor for the hormone leptin, which is only found in fat cells. The importance of this function is made clear by the leptin receptor deficiency condition, which is characterized by elevated leptin levels in blood plasma and constant hunger and overeating that result in obesity and infertility. By way of a storable transport mechanism, leptin enters the central nervous system (CNS) and binds to the long form of the leptin receptor, which is mostly found in the arcuate nucleus of the hypothalamus.

(Banks et al, 1996). Although the hypothalamus is considered to be the main target for leptin, it becoming clear that leptin has action on other tissues (Schwartz et al, 1996). Additionally, peripherally, leptin receptors are highly expressed, including the stomach, pancreases, adrenals, ovaries, and tests, where leptin has an impact on the synthesis and release of hormones like insulin, cortisol, and gonadal steroids like estradiol and progesterone in women and testosterone in males (Caprio et al, 1999; Seufert, 2004). Influence of the leptin receptor gene polymorphism rs1137101 on the onset of diabetes mellitus type 2 One of them, the (rs1137101) polymorphism, results from a non-conservative A to G substitution at codon 223, which changes the amino acid glutamine to arginine. This functional variation weakens leptin signaling by reducing leptin binding. (Quinton et al, 2001). Numerous polymorphisms have been studied, and rs1137101 has consistently been linked to a number of illnesses. Our findings shown that the LEPR gene SNP rs1137101 does not have any connection with obesity or type 2 diabetes in women's infertility. In a recent study, Dallel et al. found that Bahraini women with PCOS and control women had significantly different allele frequencies of the rs1137101 polymorphism (Yazdi et al, 2015)..They also indicated that the G allele of rs1137101 polymorphism is associated with the risk of infertility in Bahraini women, while this association was not found in Tunisians (Dallel, 2021).

Table 2. Genotype and Allele Frequency for rs1137101 in LEPR gene

Groups (each group have n=30)	Alleles, n (%)		Genotypes, n (%)			
	A	G	AA	AG	GG	
G1: Control	24(40)	36(60)	3(10)	18(60)	9(30)	
G2: Infertility with obesity	28(46.66)	32(53.33)	3(10)	22(73.33)	5(16.66)	
G3: Infertility with T2DM	28(46.66)	32(53.33)	2(6.66)	24(80)	4(13.33)	
Comparisons						
G1 vers us G2	OR(95% CI)	1.3125(0.6364 to 2.7070)	0.7619(0.3694 to 1.5714)	1(0.1851 to 5.4027)	1.833(0.6163 to 5.4535)	0.466(0.1354 to 1.6088)
	X2 / P value	0.543/0.46	0.543/0.46	0/1	1.2/0.27	1.49/0.22
G1 vers us G3	OR(95% CI)	1.3125(0.6364 to 2.7070)	0.7619(0.3694 to 1.5714)	0.6429(0.0995 to 4.1531)	2.66(0.8402 to 8.4636)	0.359(0.0968 to 1.3314)
	X2 / P value	0.543/0.46	0.543/0.46	0.218/0.64	2.85/0.09	2.45/0.12
G2 vers us G3	OR(95% CI)	1(0.4881 to 2.0489)	1(0.4881 to 2.0489)	1.55(0.2408 to 10.0495)	0.687(0.2058 to 2.2970)	1.3(0.3127 to 5.4043)
	X2 / P value	0/1	0/1	0.218/0.64	0.373/0.54	0.131/0.71

G1= Control; G2= Infertility with obesity; G3= Infertility with T2DM. OR = odd ratio; χ^2 =chi square.

CONCLUSION

Leptin receptor Q223 (rs1137101) polymorphism does not correlate with infertility in Iraqi patient women.

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